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ABSENCE OF SIMIAN VIRUS 40 IN HUMAN BRAIN TUMORS FROM NORTHERN INDIA

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Simian virus 40 (SV40), a monkey polyomavirus, was a contaminant of early poliovirus vaccines administered to millions of individuals in the 1950s and early 1960s. SV40 causes brain tumors in laboratory animals, and SV40 DNA sequences have been variably identified in human choroid plexus tumors and ependymomas. We studied the possible association between SV40 and human brain tumors in northern India, where humans have frequent contact with SV40-infected rhesus macaques. DNA from pathologic specimens from 33 ependymomas, 14 choroid plexus tumors and 18 control brain tissues (contused brain, brain metastases) was extracted and analyzed under masked conditions. We used real-time PCR to detect and quantify SV40 (T antigen) and human (*GAPDH*) DNA sequences. The SV40 PCR assay detected as few as 10 copies of SV40 DNA and had a linear range from 1×10^2 to 1×10^6 copies. SV40 DNA was detected in 1 specimen (an ependymoma). However, few SV40 DNA copies were detected in this sample (<10 copies, equivalent to <1 copy/350 cells, based on simultaneous *GAPDH* quantification), and SV40 was not detected when this sample was retested. Our findings do not support a role for SV40 in choroid plexus tumors or ependymomas from northern India.

Key words: *simian virus 40; brain tumor; India; ependymoma; choroid plexus tumor*

The monkey polyomavirus simian virus 40 (SV40) was a contaminant of early poliovirus vaccines, which were produced in kidney tissue from rhesus macaques.[1] Following the discovery of SV40 in 1960,[2] changes in vaccine preparation were soon implemented; poliovirus vaccines have been required to be free of SV40 since 1963. However, during the period of vaccine contamination (1955–1962), millions of persons, mostly children, were exposed to SV40 through large-scale poliovirus vaccination campaigns.[1]

This contamination has caused concern because SV40 induces malignancies in laboratory animals, notably brain tumors (ependymoma and choroid plexus tumor),[3,4] mesothelioma and osteosarcoma. SV40 DNA sequences have been detected in human brain tumors, including ependymomas and choroid plexus tumors, as well as other cancers.[5–8] The finding of SV40 DNA sequences in tumors from individuals too young to have received SV40-contaminated poliovirus vaccines[5–7] raises the question, so far unanswered, of whether SV40 is presently being transmitted asymptomatically in the human population. Creating further uncertainty, other laboratory studies of SV40 in human brain tumors have been largely negative.[9–12]

Humans living in northern India may be uniquely exposed to SV40 through contact with infected monkeys. SV40 infection is endemic in rhesus macaques living in this region,[13] and humans live in close contact with them. If SV40 can be transmitted among humans, as implied by the detection of SV40 DNA sequences in childhood brain tumors, then it appears likely that SV40 can be transmitted from monkeys to humans. SV40 is present in infected monkey urine,[14] and transmission to humans might occur through ingestion of contaminated food or water.

Because people living in northern India might acquire SV40 from infected rhesus macaques, we hypothesized that an association between SV40 and human cancer should be readily detectable in a study based in this region. We therefore examined brain tumor specimens from northern India for the presence of SV40 DNA.

MATERIAL AND METHODS

Tissue specimens

We retrieved archived pathologic specimens from the All India Institute of Medical Sciences, which is the major referral center for cancer treatment for New Delhi and the surrounding region, including Uttar Pradesh (east of New Delhi). We included cases of ependymoma and choroid plexus tumors diagnosed in 1991–2000. Brain tissues from individuals without primary brain tumors (brain contusion, metastasis) were included as controls and frequency-matched to cases by age (<18, ≥18 years) and year of diagnosis (1991–1995, 1996–2000).

Tissues from cases and controls had previously been fixed in formalin (for 6–8 hr) and embedded in paraffin, using standard methods. Fresh sections (10 μm) were prepared using precautions to minimize DNA contamination (*i.e.,* wiping microtome with xylene and alcohol, changing blades between specimens). An independent pathologic review of hematoxylin and eosin–stained slides confirmed the previously recorded diagnoses and demonstrated that, among cases, tumor was present in sections used for PCR. As $SV40^+$ controls, we used SV40-induced mouse tumors (CRL-2162 cells grown in SCID mice; cells from ATCC, Rockville, MD), which were excised, formalin-fixed and embedded in paraffin.

Laboratory methods

All DNA extraction and laboratory assays were performed masked to case/control status. Total DNA was extracted from tissue sections as previously described.[15] Extracted DNA was resuspended in 75 μl of TRIS EDTA (TE) and purified using the Qiagen (Hilden, Germany) DNeasy kit according to the manufacturer's instructions. Reactions replacing test specimens with diethylpyrocarbonate (DEPC) water or human DNA were included in each experiment as negative controls.

Using the well-characterized primer set sv.for3 and sv.rev,[5] amplification parameters and conditions were adapted for real-time PCR quantification of SV40 DNA. For quantitative PCR, 10 μl of extracted tissue DNA were amplified in a master mix containing

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10 mM TRIS-HCl (pH 8.0); 50 mM KCl; 200 μM each of dATP, dCTP, dGTP and dTTP; 4 mM $MgCl_2$; 5 units AmpliTaq Gold (Applied Biosystems, Foster City, CA) DNA polymerase; 0.2 × SYBR green dye (Molecular Probes, Eugene, OR) in DMSO; and 0.2 μM each primer (sv.for3 and sv.rev). The reaction plate was centrifuged briefly, and samples were amplified on an ABI (Foster City, CA) Sequence Detection System 5700 thermal cycler according to the following thermal profile: 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 12 min and 50 cycles alternating 95°C for 15 sec and 55°C for 30 sec.

Each SV40 amplification plate contained dilution replicates of SV40 DNA (ATCC 45019) at known concentrations, spanning 1×10^1 to 1×10^6 SV40 genomes/amplification reaction, which were used to generate an external standard curve for SV40 quantification. Following amplification, the normalized fluorescence (Rn) of standards was plotted by cycle number. The Rn threshold was manually selected within the linear phase of the amplification curve (usual threshold = 0.5). For each sample, the cycle threshold (C_t) was then determined as the cycle on which the curve crossed the threshold. The C_t for the standards was plotted by $\log_{10}$ input copy number, and the regression through these points (performed automatically by the Sequence Detection System 5700 software) determined the copy number for the unknown samples, based on their C_t values.

A dissociation profile of the PCR-amplified products was generated automatically by the Sequence Detection System 5700 program. Accordingly, SV40 copy number was assigned for samples generating a PCR product with denaturation temperature of 82°C, corresponding to the 105 bp amplified segment of the SV40 T-antigen gene. Nonspecific amplification products, particularly primer–dimer products, were identified by fluorescence peaks below 82°C.

PCR-amplified products were further characterized by hybridization with a biotinylated SV40 probe (GGAAAGTCCTTGGGGTCTTCTACC).[16] Briefly, 3 μl of PCR product were denatured in 0.4 N NaOH and transferred to a nylon membrane in a 96-well dot-blot format. Hybridization and detection were performed as previously described, with signal detected by autoradiogram of chemiluminescence from bound biotinylated probe.[17] A positive signal on the autoradiogram was considered confirmatory for SV40 amplification.

In parallel with SV40 PCR amplification, we used primers targeting the single-copy human gene *glyceraldehyde-6-phosphate dehydrogenase (GAPDH)* to estimate total cell number (P.E. Gravitt *et al.,* unpublished data). Briefly, PCR amplification was performed as described for SV40, substituting GAPDH1 and GAPDH2 primers, which amplify a 288 bp sequence. The *GAPDH* standard curve was produced by amplifying DNA extracted from K562 cells, diluted to yield 100,000, 20,000, 4,000, 800, 160 and 32 copies/well. Samples were considered human DNA^+ if they demonstrated peak fluorescence at 87°C. Quantification was based on interpolation from the *GAPDH* standard curve. The *GAPDH* assay had a linear range from 160 to 100,000 copies (data not shown). Total cell number was estimated by dividing the *GAPDH* copy number by 2, assuming primarily diploid genomes.

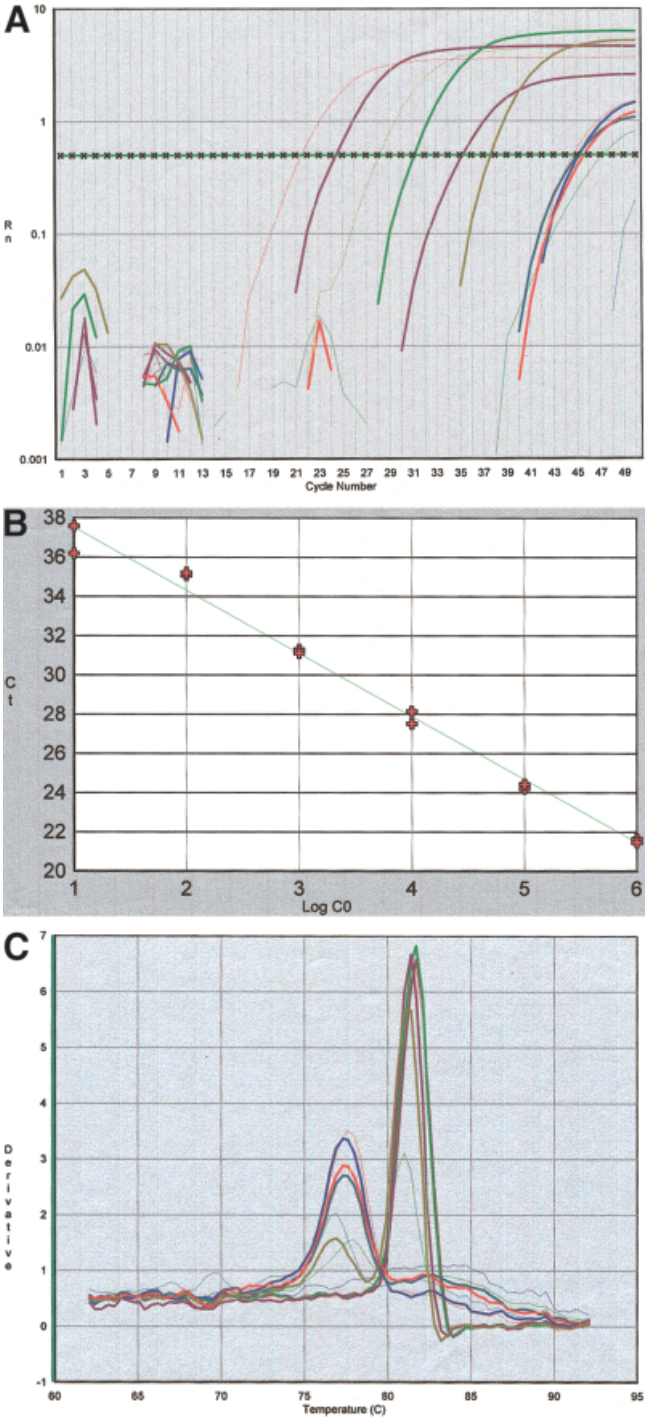

FIGURE 1 – *(a)* SV40 amplification curves. Normalized fluorescence (Rn) plotted by cycle number. Horizontal dotted line indicates the threshold, and the PCR cycle at which each amplification curve crosses the threshold determines the cycle threshold (C_t) value. Shown are amplification curves for a 10-fold dilution series of SV40 from 1×10^6 to 1×10^1 copies (with corresponding C_t values of 21.5, 24.4, 28.1, 31.1, 35.1 and 37.6), 4 negative samples (C_t values between 44.5 and 46) and a single positive sample from an ependymoma (C_t = 47.2). *(b)* Linear regression fit of C_t as a function of $\log_{10}$-transformed input SV40 copy number, from replicates of 10^6, 10^5, 10^4, 10^3, 10^2 and 10^1 copies of the SV40 standard (R^2 = 0.99). Amplification curves for 1 replicate at each copy number are shown in *(a). (c)* Denaturation temperature of PCR products obtained by the Sequence Detection System 5700 Dissociation Profile Option. The first derivative of fluorescence *vs.* temperature identifies the denaturation temperature of PCR-generated products. Results are shown for the same samples as in *(a).* The 6 samples from the SV40 dilution series show a denaturation temperature of 82°C, with corresponding peak derivatives between 5.5 and 7. The 4 negative samples have peaks indicating nonspecific PCR product at lower temperatures, around 77–77.5°C. Finally, the positive ependymoma sample has a denaturation temperature of 82°C (peak derivative of approximately 3), indicating the presence of amplified SV40 DNA sequence, as well as a secondary peak at lower temperature corresponding to nonspecific PCR product.

RESULTS

The quantitative SV40 PCR assay demonstrated a linear range from 1×10^2 to 1×10^6 copies, based on a titration series (Fig. 1*a,b*). This assay also reliably detected as few as 10 copies, though the C_t value was more variable between replicates. Amplified SV40 DNA showed a characteristic dissociation profile with a peak at 82°C (Fig. 1*c*). On the quantitative assay, 6 of 7 $SV40^+$ mouse tumors had detectable SV40, albeit at a low DNA copy number (C_t 43–48, corresponding to <10 copies, when tested in parallel with the human tissues described below). Filter hybridizations consistently detected ≥10 copies of SV40 DNA and were positive in all 7 mouse tumors (Fig. 2). All of the water and human DNA controls were consistently negative in quantitative PCR and hybridization for SV40 DNA (Fig. 2).

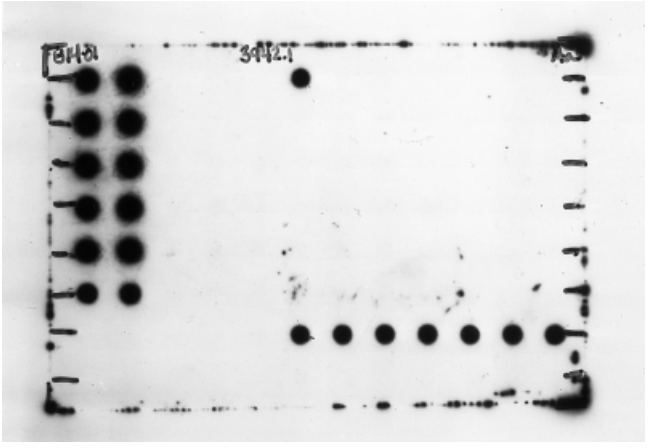

FIGURE 2 – Filter hybridization using an SV40 sequence-specific chemiluminescent probe. Results are shown in an 8-row × 12-column dot-blot format for PCR-amplified products from known standards and brain tissue specimens. In the first 2 columns, results for a dilution series are shown in duplicate: 1×10^6 copies in row 1 down to 1×10^1 copies in row 6. Row 7 (columns 6–12) shows $SV40^+$ mouse tumors (positive controls). Row 8 (columns 1–12) shows DEPC water (negative controls). The remaining 65 specimens are from the tested brain tumors and control tissues. Only 1 sample (an ependymoma, described in Results and presented in Fig. 1) showed SV40-specific hybridization (row 1, column 6).

We tested 65 human tissue specimens: 33 ependymomas (median patient age at diagnosis = 26 years, interquartile range 16–35), 14 choroid plexus tumors (14 years, 5–30) and 18 control brain tissues (31 years, 16–44). By type of specimen, median *GAPDH* copies (interquartile range) were 1,997 (784–5,163) for ependymoma, 391 (251–1,415) for choroid plexus tumors and 1,324 (402–12,181) for control brain tissues. Only 1 specimen (choroid plexus tumor) yielded <100 *GAPDH* copies.

For human tissues, a single specimen (ependymoma) was $SV40^+$ by PCR. The dissociation curve for this specimen showed a denaturation temperature at 82°C (Fig. 1*c*) similar to the positive controls, and this specimen hybridized with an internal probe on filter hybridization (Fig. 2). However, the C_t value on the quantitative SV40 PCR assay was 47.2 (<10 copies, Fig. 1*a*), indicating that little amplifiable SV40 DNA was present. Since 7,009 *GAPDH* copies were detected in the same sample, this translates into <1 copy/350 cells. Finally, in a repeat experiment, SV40 DNA did not amplify from this sample, as measured by quantitative PCR and filter hybridization.

DISCUSSION

Our results do not support a role for SV40 in human brain tumors in northern India. We detected SV40 DNA in only 1 ependymoma specimen by PCR. The copy number of SV40 DNA in this tumor was very low (<1 copy/350 cells), and the finding could not be reproduced, suggesting that this single positive result may have been due to PCR contamination. The inclusion of negative and positive tissue controls, the extraction and PCR evaluation of specimens under masked conditions and the quantification of SV40 and human cellular DNA are strengths of our study.

Like other PCR-based assays for SV40 DNA,[18] our detection method was highly sensitive, detecting as few as 10 copies per reaction mixture. Additionally, our assay, similar to others,[19,20] estimated the amount of SV40 across a linear range from 1×10^2 to 1×10^6 copies. We consider results from fixed brain tissues to be semiquantitative since they were based on an external standard (titration series). The early phase of PCR amplification kinetics is susceptible to the DNA-degrading effects of formalin fixation, so the copy number derived from the growth curves for formalin-fixed tissues may not correspond to that for a similar amount of SV40 DNA present in the titration series. Nonetheless, because it is unlikely that *GAPDH* and SV40 sequences would be differen-

TABLE I – PCR DETECTION OF SV40 IN HUMAN EPENDYMOMAS AND CHOROID PLEXUS TUMORS

Reference	Country	Number positive/ number tested (%)[1]	PCR primers[2]	PCR cycles	Tissue type[3]	Negative controls/ masking[4]	Quantification of SV40 DNA
Bergsagel *et al.*[5]	United States	E: 10/11 (91) C: 10/20 (50)	sv.for3/sv.rev	45–60	Fresh, formalin	Yes/no	No
Krainer *et al.*[10]	Austria	E: 0/10 (0)	svo.for/svo.rev (sv.for3/sv.rev nested)	45–60	Formalin	No/no	No
Martini *et al.*[6]	Italy	E: 8/11 (73) C: 5/6 (83)	pyv.for/pyv.rev	35–105	Fresh, formalin	Yes/no	No
Suzuki *et al.*[26]	Japan	E: 4/13 (31)	sv.for2/sv.rev	45	Formalin	Yes/no	No
Huang *et al.*[7]	Switzerland	E: 9/16 (56) C: 6/16 (38)	SVTAGP1/P2 SVTAGP1/P3	45	Formalin	No/no	No
Ohgaki *et al.*[12]	Finland	E: 0/10 (0) C: 0/7 (0)	SVTAGP1/P2 SVTAGP1/P3	45	ND	No/no	No
Weggen *et al.*[9]	Germany	E: 2/27 (7)	sv.for3/sv.rev LA1/LA2	40	Fresh	Yes/no	No
Reuther *et al.*[11]	Germany	E: 3/62 (5)	sv.for3/sv.rev RA1/RA2	36	Fresh, formalin	No/no	No
Present study	India	E: 0/33 (0) C: 0/14 (0)	sv.for3/sv.rev	50	Formalin	Yes/yes	Yes

[1]Entries are for ependymoma (E) and choroid plexus tumors (C).–[2]See Bergsagel *et al.*[5] and Reuther *et al.*[11] for descriptions of PCR primer pairs.–[3]Fresh, fresh frozen; formalin, formalin-fixed and paraffin-embedded; ND, not described.–[4]Negative controls, inclusion of biologic tissues as negative control; masking, documentation that both extraction of DNA and PCR were done under masked conditions.

tially affected by formalin fixation, the relative quantification of SV40 and human DNA remains valid.

In the present study, 75% of specimens yielded at least 437 *GAPDH* copies (*i.e.,* DNA roughly equivalent to 200 diploid cells). We would therefore have detected SV40 in most specimens if it had been present in at least 10 copies/200 cells (*i.e.,* 1 copy/20 cells). In SV40-induced tumors in animals, viral DNA can be found integrated into the cellular genome and SV40 T antigen is readily detected,[21] which imply the presence of at least 1 copy of viral DNA in each tumor cell. In SV40-infected human cell lines, extremely low levels of SV40 T antigen may be sufficient to induce a transformed phenotype,[22] though presumably these effects still require the presence of SV40 in each proliferating cell. If persistent SV40 infection is indeed necessary for oncogenesis, our negative results argue against an etiologic role for SV40 in human tumors.

Humans in northern India are at risk for SV40 infection through close proximity to SV40-infected rhesus macaques. A detailed ecologic study in 1959–1960 found 800,000 rhesus macaques in Uttar Pradesh, with 75% living in villages or cities, 3% in temples or railway stations and 9% along roads or canals.[23] Rhesus macaques were extensively trapped in the 1950s and 1960s, causing a population decline; but their numbers recovered rapidly following a 1978 export ban. With the destruction of woodlands for agriculture, macaques have become increasingly prevalent in towns and cities.[24] Given this ecology, human encounters with monkeys occur frequently during everyday life.

Limited data indicate that SV40 may infect individuals in this region, perhaps as a result of contacts with infected macaques. Low-titer SV40 neutralizing antibodies are present in 6–11% of the general population in Uttar Pradesh,[25] while higher-titer antibodies are found in 27% of workers in monkey export firms in Uttar Pradesh.[25] The presence of antibody, particularly at low titer, might indicate infection with another cross-reacting polyomavirus (*e.g.,* the human polyomaviruses BK and JC). However, further evidence that viral transmission between macaques and humans occurs is provided by the observation that monkeys often acquire human infections: half of rhesus adults have antibodies to measles, and 30% have antibodies to parainfluenza 3 virus.[13]

We do not know whether poliovirus vaccine was used in India in the 1950s and early 1960s, though we suspect that large-scale vaccination campaigns did not begin there until later. Nonetheless, our results on ependymoma and choroid plexus tumor are directly comparable with those from studies in the United States, Europe and Japan (Table I), where the principal exposure to SV40 is thought to have been through early poliovirus vaccines. Detection of SV40 in persons too young to have received SV40-contaminated poliovirus vaccines, taken at face value, requires ongoing transmission of SV40 among humans.[5–7] Along these lines, the presence of SV40-infected macaques in India provides a plausible reservoir for continued reintroduction of this virus into the human population, and SV40 might be transmitted among humans in India as elsewhere.

Reconciling the conflicting results of these studies thus requires a careful review of their methods and findings (Table I). Our negative results are supported by 3 studies from Austria and Germany.[9–11] A Finnish study was also negative,[12] but this was attributed by the investigators to a lack of poliovirus vaccine contamination in Finland. In our study and the Austrian study,[10] all of the specimens were fixed in formalin, which could have degraded the DNA. However, few tumors in the German studies were $SV40^+$, despite use of fresh frozen specimens,[9,11] and other studies using only formalin-fixed tissues yielded positive results.[7,26] Our quantitative testing for the human gene *GAPDH* indicated that most of our specimens had adequate PCR-amplifiable DNA.

It is important to consider the alternative possibility that the positive results in some studies described in Table I were due to laboratory artifact.[5–7,26] SV40 DNA sequences are present in over 200 cloning vectors used by laboratories worldwide,[27] which might lead to PCR contamination. In this regard, 1 positive study did not include negative control tissues,[7] and none described masked extraction and testing of specimens. In 1 study,[6] SV40 DNA was found in a wide range of tumor types and normal cells, and almost all specimens also had detectable BK virus, calling into question the specificity of testing. Finally, no study quantified the amount of SV40 DNA present in tumor specimens. One study used repeated PCR cycling to increase sensitivity,[6] but our results indicate that highly sensitive assays (including our assay) can identify extremely low levels of SV40 that could arise from PCR contamination.

Other evidence also points away from SV40 as a cause of human brain tumors. A previous study of cancer patients in northern India found SV40 antibodies in only 1 of 13 patients (8%) with CNS tumors,[28] similar to the seroprevalence in other cancer patients and the general population.[25,28] Antibody titers were generally low and may have been due to cross-reactivity with another virus (*e.g.,* BK or JC). Long-term follow-up of children exposed to SV40-contaminated poliovirus vaccines in the United States and Europe has not revealed excess risk for brain tumors, specifically including ependymoma and choroid plexus tumor.[29,30] Finally, no brain tumors were observed during 35 years of follow-up of neonates given poliovirus vaccine with live SV40,[31,32] despite the documented exposure occurring in a period of marked immune immaturity.

In conclusion, our results do not support a role for SV40 in the etiology of brain tumors. Given concerns regarding possible laboratory contamination with SV40, future laboratory studies of SV40 in human cancer should include appropriate tissue controls, blinding and quantification of SV40 in positive specimens.

REFERENCES

1. Shah K, Nathanson N. Human exposure to SV40: review and comment. Am J Epidemiol 1976;103:1–12.
2. Sweet BH, Hilleman MR. The vacuolating virus, SV40. Proc Soc Exp Biol Med 1960;105:420–7.
3. Kirschstein RL, Gerber P. Ependymomas produced after intracerebral inoculation of SV40 into new-born hamsters. Nature 1962;195:299–300.
4. Rabson AS, O'Conor GT, Kirschstein RL, Branigan WJ. Papillary ependymomas produced in *Rattus [Mastomys] natalensis* inoculated with vacuolating virus (SV40). J Natl Cancer Inst 1962;29:765–87.
5. Bergsagel DJ, Finegold MJ, Butel JS, Kupsky WJ, Garcea RL. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N Engl J Med 1992;326:988–93.
6. Martini F, Iaccheri L, Lazzarin L, Carinci P, Corallini A, Gerosa M, Iuzzolino P, Barbanti-Brodano G, Tognon M. SV40 early region and large T antigen in human brain tumors, peripheral blood cells, and sperm fluids from healthy individuals. Cancer Res 1996;56:4820–5.
7. Huang H, Reis R, Yonekawa Y, Lopes JM, Kleihues P, Ohgaki H. Identification in human brain tumors of DNA sequences specific for SV40 large T antigen. Brain Pathol 1999;9:33–44.
8. Shah KV. Polyoma viruses (JC virus, BK virus, and simian virus 40) and human cancer. In: Goedert JJ, ed. Infectious causes of cancer: targets for intervention. Totowa, NJ: Humana, 2000. 461–74.
9. Weggen S, Bayer TA, von Deimling A, Reifenberger G, von Schweinitz D, Wiestler OD, Pietsch T. Low frequency of SV40, JC and BK polyomavirus sequences in human medulloblastomas, meningiomas and ependymomas. Brain Pathol 2000;10:85–92.
10. Krainer M, Schenk T, Zielinski CC, Müller C. Failure to confirm presence of SV40 sequences in human tumours. Eur J Cancer 1995;31A:1893.
11. Reuther FJ, Löhler J, Herms J, Hugo HH, Schindler C, Leithäuser F, Melzner I, Möller P, Scheil S. Low incidence of SV40-like sequences in ependymal tumours. J Pathol 2001;195:580–5.
12. Ohgaki H, Huang H, Haltia M, Vainio H, Kleihues P. Cell and molecular biology of simian virus 40: implications for human infections and disease. J Natl Cancer Inst 2000;92:495–6.
13. Shah KV, Southwick CH. Prevalence of antibodies to certain viruses

in sera of free-living rhesus and of captive monkeys. Ind J Med Res 1965;53:488–500.
14. Shah KV, Willard S, Meyers RE, Hess DM, Digiacomo R. Experimental infection of rhesus with simian virus 40 (SV40). Proc Soc Exp Biol Med 1969;130:196–203.
15. Bauer HM, Greer CE, Manos MM. Determination of genital human papillomavirus infection by consensus PCR amplification. In: Herrington CS, McGee JO, eds. Diagnostic molecular pathology: a practical approach. Oxford: Oxford University Press, 1992;131–152.
16. Carbone M, Pass HI, Rizzo P, Marinetti M, Di Muzio M, Mew DJY, Levine AS, Procopio A. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994;9:1781–90.
17. Shah KV, Daniel RW, Strickler HD, Goedert JJ. Investigation of human urine for genomic sequences of the primate polyomaviruses simian virus 40, BK virus, and JC virus. J Infect Dis 1997;176:1618–21.
18. Strickler HD, International SV40 Working Group. A multicenter evaluation of assays for detection of SV40 DNA and results in masked mesothelioma specimens. Cancer Epidemiol Biomarkers Prev 2001; 10:523–32.
19. Shi L, Ho J, Norling LA, Roy M, Xu Y. A real time quantitative PCR-based method for the detection and quantification of simian virus 40. Biologicals 1999;27:241–52.
20. Gordon GJ, Chen C-J, Jaklitsch MT, Richards WG, Sugarbaker DJ, Bueno R. Detection and quantification of SV40 large T-antigen DNA in mesothelioma tissues and cell lines. Oncol Rep 2002;9:631–4.
21. Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. Am J Pathol 1993;142:1524–33.
22. Waheed I, Guo ZS, Chen GA, Weiser TS, Nguyen DM, Schrump DS. Antisense to SV40 early gene region induces growth arrest and apoptosis in T-antigen-positive human pleural mesothelioma cells. Cancer Res 1999;59:6068–73.
23. Southwick CH, Beg MA, Siddiqi MR. A population survey of rhesus monkeys in northern India. Ecology 1965;42:698–710.
24. Southwick CH, Siddiqi MF. Primate commensalism: the rhesus monkey in India. Rev Ecol 1994;49:223–31.
25. Shah KV. Neutralizing antibodies to simian virus 40 (SV40) in human sera from India. Proc Soc Exp Biol Med 1966;121:303–7.
26. Suzuki SO, Mizoguchi M, Iwaki T. Detection of SV40 T antigen genome in human gliomas. Brain Tumor Pathol 1997;14:125–9.
27. Völter C, zur Hausen H, Alber D, de Villiers EM. A broad spectrum PCR method for the detection of polyomaviruses and avoidance of contamination by cloning vectors. Dev Biol Stand 1998;94:137–42.
28. Shah KV. Investigation of human malignant tumors in India for simian virus 40 etiology. J Natl Cancer Inst 1969;42:139–45.
29. Strickler HD, Rosenberg PS, Devesa SS, Hertel J, Fraumeni JF Jr, Goedert JJ. Contamination of poliovirus vaccines with simian virus 40 (1955–1963) and subsequent cancer rates. JAMA 1998;279:292–5.
30. Geissler E. SV40 and human brain tumors. Prog Med Virol 1990;37: 211–22.
31. Carroll-Pankhurst C, Engels EA, Strickler HD, Goedert JJ, Wagner J, Mortimer EA Jr. Thirty-five year mortality following receipt of SV40-contaminated polio vaccine during the neonatal period. Br J Cancer 2001;85:1295–7.
32. Mortimer EA, Lepow ML, Gold E, Robbins FC, Burton GJ, Fraumeni JF Jr. Long-term follow-up of persons inadvertently inoculated with SV40 as neonates. N Engl J Med 1981;305:1517–8.